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POSSIBILITIES OF APPLICATION OF NATURAL ZEOLITES IN STORED WHEAT GRAIN PROTECTION AGAINST PEST INSECTS

MOGUĆNOSTI PRIMENE PRIRODNOG ZEOLITA U ZAŠTITI USKLADIŠTENE PŠENICE OD ŠTETNIH INSEKATA

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ABSTRACT

Natural zeolites are widely used in agriculture, but they also have an insecticidal potential in control of storage insects S. oryzae, R. dominica and T. castaneum. Their efficacy depends on relative air humidity, exposition and powder properties (Natural zeolite-NZ, Modified natural zeolite-MNZ and Fine Granulated Natural zeolite-FGNZ). NZ and MNZ applied at rate 0.25-0.75 g/kg of wheat, at 24ºC and 45±5% r.h., are >90% efficient against S. oryzae and T. castaneum after 21 days of exposition, and against R. dominica 23-73%, when they also have the highest impact on offspring reduction. At a higher r.h. the efficacy against parents (50-55%) and offspring (60±5%) is significantly lower, particularly for MNZ. For treatment of wheat with 1 g/kg NZ and FGNZ, the highest efficacy is after 21 days of exposition (>95% and 79%), and so is the offspring reduction (82 and >90%), which indicates that the use of natural zeolites can provide a successful protection of stored wheat grains from pest insects attack.

Keywords: *wheat grain, storage insects, natural zeolite, pest control.*

REZIME

Primena insekticida je zbog rezidua u hrani i rezistentnosti skladišnih insekata pod sve većom restrikcijom. Među alternativnim merama se po efektivnosti i bezbednosti izdvaja diatomejska zemlja – DZ, kao inertno prašivo. Međutim, noviji rezultati istraživanja pokazuju da i prirodni zeoliti, kao najsličniji DZ, poseduju značajan insekticidni potencijal u suzbijanju skladišnih insekata, na primer: Sitophilus oryzae, Rhyzopertha dominica i Tribolium castaneum, uz isticanje visoke zavisnosti efikasnosti od relativne vlažnosti vazduha, dužine izlaganja i osobina prašiva (Prirodni zeolit – PZ, Modifikovani prirodni zeolit – MPZ i Prirodni zeolit finije granulacije – PZFG). Prašiva PZ i MPZ primenjena u količini 0,25 0,50 i 0,75 g/kg pšenice, na 24ºC i 45±5% r.v.v, su za S. oryzae i T. castaneum posle 21 dan izlaganja efikasni >90%, a za R. dominica 23-73%, kada je i najveći uticaj na redukciju potomstva. Prašivo PZ visoku redukciju potomstva (90%) ostvaruje samo sa količinom 0,75 g/kg. Sva prašiva su pri 50-55% r.v. (za roditelje) i 60±5% (za potomstvo) značajno manje efikasna, a naročito MPZ. Najvišu efikasnost, >95% i 79% su ostvarili PZ i PZFG posle 21 dan kontakta S. oryzae i T. castaneum, odnosno R. dominica, sa pšenicom tretiranom sa 1 g/kg, dok je najviša redukcija potomstva ostvarena posle 21 dan ekspozicije roditelja, kod T. castaneum i R. dominica > 90%, a kod S. oryzae 85 i 82%. Rezultati istraživanja pokazuju da prašiva na bazi prirodnog zeolita, kada se primene u količinama kao i preparati na bazi diatomejske zemlje, mogu uspešno da zaštite uskladištenu pšenicu od napada najvažnijih vrsta štetnih insekata.

Ključne reči: *pšenica u zrnu, skladišni insekti, prirodni zeolit, suzbijanje.*

INTRODUCTION

Globally harmonized regulations in the world food trade require the use of more efficient and cheaper measures or methods in protection from pest organisms, without side effects on consumers or beneficial organisms in agro ecosystem and the environment. Therefore, the contemporary protection of stored plant products from pests, as final and very important segment of agricultural production, is oriented towards preventive action and monitoring, while chemical control measures are taken only when inevitable (Hagstrum and Flin, 1996; Kljajić, 2008; Lazarides, 2009; Kljajić and Andrić, 2010; Vujošević et al., 2010).

In recent years, the use of contact insecticides and fumigants, as an essential elements of chemical control of storage insects, is under increasing restriction due to the presence of harmful residues in food and resistance of storage insects (Kljajić and Perić, 2005; Collins, 2006). Hence, further activities aim at introduction of alternative protection methods, among of which inert dusts, earlier described as sorptive dusts, stand for efficacy and safety (Ebeling, 1971). Among all inert dusts the best known is diatomaceous earth, and some of its products are used in practice worldwide (Golob, 1997; Korunić,1998; Subramanyam and Roesli, 2000). The efficacy of natural zeolites and other inert dusts, besides species and developmental stage of storage insects (Korunić, 1998; Athanassiou et al., 2007; Kljajić et al., 2010a,b) is also significantly affected by environmental conditions, above all temperature and humidity of air and substrate (Fields and Korunić, 2000; Arthur, 2001; Athanassiou et al., 2005), exposition duration (Fields et al., 2003; Kljajić et al., 2010a), also by the content of silicium dioxide (Korunić, 1997 and 1998; Kljajić et al., 2009b), as well as by shape and size of particles present in the product (Subramanyam and Roesli, 2000; Vayias et al., 2009).

Natural zeolites (alkaline aluminium silicates) are, according to their physical properties, the most similar to diatomaceous earth, so they can be classified in the same group – dusts with natural silicates (Subramanyam and Roesli, 2000). They are widely used in agriculture, particularly for improvement of soil properties and yield increase of crops, but also as a food supplement for farm animals because they reduce the presence of mycotoxins (Reháková et al., 2004; Đorđević and Dinić, 2007). However, the results of recent studies also point to significant insecticidal potential of natural zeolite in control of storage pests, such as: rice weevil *Sitophilus oryzae* (L.), lesser grain borer *Rhyzopertha dominica* (F.) and red flour beetle *Tribolium castaneum* (Herbst), yet underlying high dependence of demonstrated efficacy on relative air humidity, exposure duration and dust properties (Kljajić et al., 2007 and 2010 a,b).

The aim of this paper was to consider from different perspectives the possibility of a more massive use of natural zeolite in protection of stored wheat from pest insects, particularly accounting for the food safety.

MATERIAL

Inert dusts

The use of inert dusts in protection of stored plant products was known in the time of ancient civilizations. However, it was only in the last twenty years, because of difficulties with synthetic insecticides use, that they became the subject of the important researches. Inert dusts, according to their properties, can be divided into four groups: 1) the first group of dusts include: clay, sand, ashes created by burning of various plants and volcanic dust 2) in this group fall minerals such as dolomite, magnesite, calcium carbonate and various phosphates, 3) dusts that contain synthetic silicates and 4) dusts containing natural silicates, where diatomaceous earth and natural zeolite belong. Among all inert dusts, the most favorable properties have powders that contain natural silicates, and diatomaceous earth (DE) has the highest practical significance and in many countries it is an integral part of program for stored products protection from harmful insects (Subramanyam and Roesli, 2000).

Inert dusts have a range of advantages over synthetic insecticides: low mammal toxicity (except possible damage of respiratory organs in people who are directly exposed to dust application), they minor affect the quality of wheat and its products and they are environmentally friendly. On the other hand, they act slower than insecticides, and the death of storage insects appears as a consequence of: 1) spiracles blocking 2) water loss for cuticle damage 3) binding of water from cuticle 4) suffocation provoked by small particles inhalation (intake) and 5) binding of lipids from epicuticular layer. It is important to point out that because of independent action of these substances from insect metabolic functions, it is considered that occurrence of insect resistance is not possible, which is a great advantage compared to recently used contact insecticides and fumigants (Ebeling, 1971; Golob, 1997; Korunić,1998; Subramanyam and Roesli, 2000; Fields and White, 2002).

Insecticide potential of natural zeolites and other inert dusts, directly depends on their physical and chemical properties (structure, SiO_2 content, size and presence of particles, pH values, sorptive capacity and geographical origin), and also on their impact on mass density of stored wheat, particles subsidence and binding to wheat surface (Subramanyam and Roesli, 2000). Dusts with amorphous structure, like diatomaceous earth (DE), have proven, for now, to be significantly more efficient than dusts with crystal structure. For example, under laboratory conditions (relative air humidity 45±5% and temperature 24±1ºC) product on the basis of DE, Protect-It, applied at rates 0.15, 0.20 and 0.30 g/kg after 7 days of exposure in treated wheat caused 69, 25 and 83% mortality, respectively, while Natural zeolite (NZ) at the rate of 0.75 g/kg caused 86, 25 i 53% mortality in *S. oryzae*, *R dominica* and *T. castaneum* (Kljajić et al., 2010a). At this point, it is important to point out that most of the products on the basis of DE which are available on the world market contain over 85% of amorphous SiO_2, and many commercial products also contain materials which improve their efficacy. Protect-It contains 10% of silica gel, Insecto 10% of additives, and PyriSec 1,2% of natural pyrethrin (Subramanyam and Roesli, 2000). The content of SiO_2 in dusts is in a positive correlation with the efficacy, which for *S. oryzae* and *T. castaneum* was confirmed by Korunić (1997) and for *Acanthoscelides obtectus* (Say) by Kljajić et al. (2009b). Researches with different samples of natural zeolite and DE originating from Serbia also showed that SiO_2 content affects the efficacy. For example, after 7 days of exposure of *S. oryzae* to the wheat treated with 0.75 g/kg , efficacy of DE dusts (containing 78.8% and 63.1% SiO_2) was 72 and 51%, and for *T. castaneum* it was 47 and 22%, while the efficacy of dusts based on natural zeolite (NZ and MNZ-Modified natural zeolite containing 63-68% SiO_2) was 31 and 14% for *S. oryzae*, and 9 and 0% for *T. castaneum* (Andrić et al., unpublished data).

Size and percent of presence of particles are important parameters not only for the efficacy, but also for the safety of people applying them. Researches showed that dusts with particles sized < 45 µm have significantly higher efficacy than dusts with particles sized 45-150µm (Vayias et al., 2009), and particularly products with particles sized 5-15µm (Korunić, 1997 and 1998). However, small particles are potentially more hazardous to people who apply them, and therefore particles < 5 µm, particularly those < 1 µm may cause serious health problems in humans, primarily in the respiratory system (Subramanyam and Roesli, 2000). For dusts based on natural zeolite it was also recorded that the size of particles significantly affects the efficacy. For example, NZ dusts (particle size >53µm - 3.5%; 28-13µm - 28.8%) and Fine Granulated Natural Zeolite (FGNZ) (particle size >53µm - 1.3%; 28-13µm - 31.1%) in all experimental variants are significantly more efficient than MNZ (particle size >53µm - 30.0%; 28-13µm - 14.3%). Particle size distribution of sorptive dusts water suspensions (in the 0.6 nm - 6 µm diameter range) was determined using Zeta-sizer Nano ZS with 633 nm HE-Ne laser (Malvern, UK) and average diameters ranged from 579 to 1080 nm for all dusts. These research results suggest that the procedure of dust preparation itself is very important not only from efficacy perspective, but also from a perspective of people who apply them (Kljajić et al., 2010b; Andrić et al., unpublished data).

Properties of natural zeolites

The products based on natural zeolite are on positive FDA (Food and Drugs Association of USA) list and GRAS (Generally Recognized As Safe) list. Natural zeolite contains mineral clinoptilolite at the highest percentage (80-90%), it has crystal structure and negative charge. Due to these properties, it has a distinct ability of an ion exchange and in humans and animals it links (adsorbs) different toxic substances. In animal digestive tract it adsorbs mycotoxins originating from consumed food. Adsorption index for aflatoxin B_1 is over 90%, aflatoxin B_2 and G_2 over 80%, for zearalenone 60%, T_2-toxin 40% and ochratoxin 35% (Tomašević et al., 2001). Natural zeolite mostly contains following oxides: silicium dioxide (63-68%) and aluminium trioksid (11-14%), the content of other oxides is < 2.5%, while the content of heavy metals is < 40 ppm (Table 1).

Table 1. Chemical analysys of Fine Granulated Natural Zeolite (FGNZ) originating from Serbia

Compound	Content (%)
SiO_2	65.69
Al_2O_3	14.03
K_2O	1.39
Na_2O	1.41
MgO	1.09
CaO	3.57
Fe_2O_3	2.34
TiO_2	0.17
Heavy metals	Content (ppm)
Cd	< 5
Pb	< 30
Sb	< 30
Cu	< 30
Zn	< 40
As	< 20
Ignition loss	10.29%
CEC mEq/100 g*	129.56

*CEC Cation Exchange Capacity

DISCUSSION

Susceptibility of storage insects to natural zeolite

Storage insects express different susceptibility to inert dusts due to morphological, physiological and ecological characteristics of each species. All insect characteristics which affect the efficacy of dusts are in connection with a mode by which insects sustain optimal content of water in the organism, because it has been confirmed that insects with thinner and gentler wax layer are more susceptible to inert dusts, as insects whose body surface is smaller. Also, it was found that insects from different parts of the world show different susceptibility to dusts (Golob, 1997; Korunić, 1998; Subramanyam and Roesli, 2000; Vayias et al., 2006). Among the representatives of Coleoptera order the most susceptible to diatomaceous earth are species from the genus *Cryptolestes*, somewhat less from *Sitophilus* and *Oryzaephilus* genus, while *R. dominica* and species from the genus *Tribolium* are the least susceptible (Korunić, 1997; Arthur, 2001; Athanassiou et al., 2007). Researches with natural zeolite have also confirmed these observations, for application of NZ dusts at the rate of 0.25 g/kg wheat in grain, after 14 days of exposure recorded mortality was 100% for *S. oryzae*, 20% for *R. dominica* 20% and 79% for *T. castaneum*. Therefore, this fact should be borne in mind when making a decision about application rate for natural zeolite (Kljajić et al., 2010a).

Factors affecting insecticidal potential of natural zeolite

It is well known that storage insects are adapted to living under conditions of low relative air humidity and low water content in the substrate, and because of this a minimal water losses from insects body are hard to recover. It has been found that insects death occurs when they lose 60% of water or around 30% of body mass, which, because of abrasion of insect wax cuticle layer, can be provoked by inert dusts application. The increase in relative air or substrate humidity significantly reduces the efficacy of inert dusts, which can be explained by high sorptivity of inert dusts and binding of water from air or substrate. Also, under conditions of increased humidity, storage insects recover water losses caused by dust action faster, for what their efficacy may fail (Ebeling, 1971; Subramanyam and Roesli, 2000). Testing of efficacy of dusts based on natural zeolite under different conditions of relative air humidity (r.h.) showed significantly lower efficacy at higher r.h. values, particularly at shorter exposition. NZ and MNZ dusts applied at rate 0.75 g/kg after 14 days of exposition are 1.3 and 2.3 less efficient against *S. oryzae* and 1.3 i 10.3 against *T. castaneum* at 50-55% r.h. for parents exposition and 60-65% r.h. during progeny production, compared to 45±5% r.h during the whole experiment (Kljajić et al., 2010a; Andrić et al., unpublished data).

An important factor for efficacy of inert dusts is temperature, because at higher temperatures insects lose water more rapidly, and dusts efficacy is significantly increased (Subramanyam and Roesli, 2000; Athanassiou et al., 2005). For example, Dowdy (1999) after 24 h recovery of *T. castaneum* adults previously exposed 15 and 30 minutes to wheat treated with Insecto dust at 34°C observed efficacy of around 9%, while for the same exposure intervals at 50ºC, it was 27% and 62%, respectively.

Besides the above mentioned, other factors can also significantly affect insecticidal potential and efficacy of inert dusts, such as, for example, type of substrate, because it is known that maize and bean grains contain more lipids than wheat and have higher absorption and dust inactivation power (Subramanyam and Roesli, 2000). Haryadi et al. (1994) under conditions of extremely high r.h. (75-85%) found that the efficacy of natural zeolite applied at the rate of 50 g/kg maize was 100% even after 12 weeks of storage. The same authors report that germination of treated seeds was 96.2% and of untreated maize seeds 72%. Also, the presence of broken grains, a higher percentage of impurities in grain mass can significantly reduce the efficacy of inert dusts (Athanassiou, 2006). Kljajić et al. (2009a) tested the efficacy of DE samples originating from Serbia, under laboratory conditions, against *Plodia interpunctella* (Hübner) larvas in treated maize consisted of damaged and whole grains. It was found that the efficacy of dusts DE S-1 and DE S-2 applied at rate 1 g/kg after 7 days of exposure in damaged maize grains is 60% and 41%, and in whole maize grains 98% and 90%, respectively. For this reason, all the mentioned facts should be borne in mind when deciding about inert dusts application rate.

Concerning that natural zeolite, as other inert dusts also, has a slower action on storage insects than synthetic insecticides, it can be concluded that exposition duration is one more, very important factor, which affects the efficacy. Summarizing all results published to the day present, it can be concluded that most dusts realize high efficacy after 14 or 21 days of exposure (Korunić, 1997; Fields et al., 2003; Kljajić et al., 2010a). For example, dusts NZ, FGNZ and MNZ applied at rate 1 g/kg of wheat after 7 days exposure of *S. oryzae* realized 35, 40 and 14% efficacy, and after 21 days of exposure 96, 94 and 34%, respectively (Kljajić et al., 2010b; Andrić et al., unpublished data).

Subramanyam and Roesli (2000) report that prevention of massive progeny production in substrate treated with inert dusts, as indirect action, is of higher importance than direct action on parents. Kljajić et al. (2010a) under laboratory conditions (24±1ºC and 45±5% r.h) after 21 days of exposure of parents to the wheat treated with NZ at rate 0.25-0.75 g/kg recorded progeny inhibition within the range 60-95% for *S. oryzae*, 76-89% for *R. dominica* and 64-90% for *T. castaneum*. On the other hand, under conditions of increased humidity (50-55% r.h. during exposure of parents and 60-65% r.h. during progeny production) wheat treatment with FGNZ at rate 0.50-1.0 g/kg and 21 days of parents exposition resulted in progeny inhibition within the range of 51-85% for *S. oryzae*, 80-96% for *R. dominica* and 87-98% for *T. castaneum* (Kljajić et al., 2010b).

As the above mentioned, the efficacy of natural zeolites and other inert dusts depends on several factors, therefore application rates should be chosen accordingly. Products on the basis of diatomaceous earth used for stored wheat protection from storage insects are usually applied at rates ranging from 0.5 to 1.5g/kg (Subramanyam and Roesli, 2000). Based on the existing research results, in conditions of lower air (45±5%) and wheat humidity, the application of NZ at the rate of 0.75g/kg ensures high efficacy against *S. oryzae*, *R. dominica* and *T. castaneum*, while in conditions of increased humidity (55-65%) it is necessary to apply NZ at the rate of 1.0-1.5g/kg. In the light of these facts, it can be concluded that by application of NZ dust at similar rates as diatomaceous earth, stored wheat can be successfully protected, with the annotation that treated wheat should not be manipulated for 20 days.

Table 2. Efficacy of natural zeolites in treated wheat and maize against adults of storage insects under different application conditions

Dust (g/kg)	Substrate (t°C and r.h. %)	Test insect	Efficacy (%)	IR* (%)	Source
NZ 0.75	Wheat 24±1°C	*S. oryzae* *R. dominica* *T. castaneum*	100 73.7 100	95.0 88.8 90.3	Kljajić et al. (2010a)
MNZ 0.75	45±5%	*S. oryzae* *R. dominica* *T. castaneum*	98.9 43.7 100	80.0 76.0 80.6	
FGNZ 1.0	Wheat 24±1ºC 55-65%	*S. oryzae* *R. dominica* *T. castaneum*	94.0 78.7 100	85.1 96.1 98.8	Kljajić et al. (2010b)
NZ 1.0	Wheat 24±1°C	*S. oryzae* *T. castaneum*	96.0 100	81.8 96.5	Andrić et al. (unpublished data)
MNZ 1.0	55-65%	*S. oryzae* *T. castaneum*	45.0 28.0	62.0 71.3	
NZ 50.0 40.0	Maize 27-30°C 75-85%	*S. zeamais*	100 -	- 70.1	Haryadi et al. (1994)

* - Inhibition rate in F_1 generation

Possibilities of practical use of natural zeolite

Use of natural zeolite as inert dust can positively affect safety of produced food, concerning that it is one of essential requirements in designing protection measures from pest insects. The advantages of the use of dusts based on natural zeolite over synthetic insecticides are multiple, yet the most important one is that they are natural materials with a very low mammal toxicity, they leave no harmful residues in food and are environmentally friendly. Also, the use of natural zeolite in wheat intended for farm animal feed inhibits the development of microorganisms, referrenig to prevention of mycotoxin production, which stimulates the growth and health of animals.

The negative impact of natural zeolite and other inert dusts reflects in the reduction of hectoliter grain mass in storage, and in high risk of possible damage to mills and other facilities in storage houses, which is a consequence of powder abrasiveness when applied in larger quantities. Recently, this problem was mitigated by substitution of older formulations with application rate over 5 g/kg, with new formulations, highly efficient even at rate of 0.5-1.5 g/kg (Korunić et al., 1996; Korunić et al., 1998; Subramanyam and Roesli, 2000).

Aiming at the reduction of application rates and the efficacy increase, the researches of application of dusts in interaction with high temperature (for example 50ºC) and in combination with natural products, such as: essential oils, entomopathogenic fungi or with newer insecticides as spinosad and abamectin (Dowdy, 1999; Athanassiou and Korunić 2007; Chintzoglou et al., 2008) are being conducted. However, for now, the biggest opportunity for a more significant practical use of natural zeolite in stored wheat protection is in organic production and in food for farm animals.

CONCLUSION

The efficacy of natural zeolites and other inert dusts, besides species and developmental stage of storage insects is also significantly affected by environmental conditions, above all temperature and humidity of air and substrate, exposition duration, also by the content of silicon dioxide, as well as by shape and size of particles present in the product.

Based on the existing research results, in conditions of lower air (45±5%) and wheat humidity, the application of only NZ at the rate of 0.75 g/kg ensures high efficacy against *S. oryzae*, *R. dominica* and *T. castaneum*, while in conditions of increased humidity (55-65%) it is necessary to apply NZ at the rate of 1.0-1.5 g/kg. In the light of these facts, it can be concluded that by application of NZ dust at similar rates as diatomaceous earth, stored wheat can be successfully protected, with the annotation that treated wheat should not be manipulated for 20 days.

The biggest opportunity for a more significant practical use of natural zeolite in stored wheat protection is in organic production and in food for farm animals.

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